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Direct Determination by Raman Scattering of the Conformation of the Choline Group in Phospholipid Bilayers†

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ABSTRACT: For clarification of the assignments of the vibrational modes of the choline group, Raman spectra of choline iodides selectively deuterated at three different positions were investigated. The isotope shifts of the C–N stretching vibrations suggested that they are conformation sensitive. When the Raman spectra of choline chloride, carbamoylcholine iodide, carbamoylcholine chloride, and methoxycarbonylcholine iodide are compared with the crystal structures of these compounds, a correlation between the vibrational frequency and the conformation of the O–C–C–N^+ backbone could be established. The Raman bands attributed to the "totally" symmetric stretching (ν_1) and symmetric stretching vibrations (ν_2) of the C–N bonds of the quaternary ammonium group appeared at about 720 cm^{-1} and about 870 cm^{-1}, respectively, for the gauche conformation of the O–C–C–N^+ backbone, and in the trans conformation, they shifted to about 770 cm^{-1} (ν_1) and about 910 cm^{-1} (ν_2), respectively. On the basis of this correlation and from measurements of phosphatidylcholine and sphingomyelin bilayers, it was concluded that most of the choline groups in both bilayers take the gauche conformation not only in the solid state but also in the gel and liquid-crystalline states. These data represent the first direct evidence that a gauche conformation for the O–C–C–N^+ bond is preferred in the gel and liquid-crystalline states. These key bands, especially the ν_1 band, are a powerful tool to study the conformation of the choline group in situ not only in the membrane field but also in the neuroscience in connection with acetylcholine.

The choline group is an important chemical substituent in biological systems. It is found in lipids as polar head groups of phosphatidylcholine and sphingomyelin as well as in the neurotransmitter as a part of acetylcholine. The crystal structures of both acetylcholine (Jagner & Jensen, 1977, and related references in it) and the phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (Pearson & Pascher, 1979) are now known. It is relevant to physical studies on lipid membranes to know if these structures are retained in other states. For example, lipid bilayers of biomembranes are in the liquid-crystalline state under many biological conditions. It is difficult, however, to determine the molecular structures of the lipids in the liquid-crystalline state.

Several techniques such as neutron scattering, X-ray scattering, and deuterium and phosphorus NMR[1] can yield information on the positions or order parameters of various segments, but they cannot give direct information on bond conformations. In contrast, vibrational spectroscopy, especially Raman scattering, is a powerful method for such an investigation. Raman bands attributed to skeletal vibrations are relatively strong and correlate closely with the conformation. Furthermore, Raman scattering can be observed in the solid, gel, and liquid-crystalline states. A good example is the hydrocarbon chain region of lipids where Raman bands at 1066, 1130, and 1089 cm^{-1} are observed. The former two bands were assigned to the C–C stretching vibration in the all-trans conformation, and the last one was assigned to the C–C stretching vibration in the presence of the gauche conformations (Lippert & Peticolas, 1971). These assignments have been useful for the investigation of the phase transitions of lipid bilayers. In spite of its potential usefulness, the contribution of Raman spectroscopy to structural studies of the polar head groups of phospholipids has been limited due to the lack of a Raman band sensitive to the conformation. In this work, it will be shown that the Raman bands assigned to the C–N stretching vibrations are sensitive to the conformation of the choline group and that because of its strong intensity, one of these bands is appropriate for the study of the structure of the choline group in situ. Although applications are presented only for phosphatidylcholine and sphingomyelin bilayers, this method has general significance for any investigation into a molecule containing a choline group.

Materials and Methods

Selectively deuterated choline iodides and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine were kindly provided by Dr. J. Seelig. They were synthesized according to reported

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[1] Abbreviations used: ChCl, choline chloride; CarChI, carbamoylcholine iodide; CarChCl, carbamoylcholine chloride; MetChI, methoxycarbonylcholine iodide; ^1H NMR, proton magnetic resonance; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance.

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methods (Gally et al., 1975). Nondeuterated and deuterated methoxycarbonylcholines were synthesized by reacting *N,N*-dimethylaminoethanol with methyl chloroformate followed by methylation with CH_3I and CD_3I, respectively (Vieler & Galsomias, 1968). Nondeuterated choline iodide, choline chloride, carbamoylcholine chloride, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine were purchased from Fluka AG. Sphingomyelin (from bovine brain) was obtained from Sigma Chemical Co., and tetramethylammonium bromide, ethyltrimethylammonium iodide, and phosphocholine–calcium chloride were purchased from Nakarai Chemicals Ltd. Carbamoylcholine iodide was obtained by mixing almost saturated aqueous solutions of carbamoylcholine chloride and potassium iodide (Jensen, 1975). The compounds measured in solid were recrystallized in the same manner as was used for the crystal analyses.

Raman spectra were recorded on a Spex spectrometer Ramalog 5, a JRS-400 laser Raman spectrometer (Institute for Protein Research, Osaka University), and a Kawaguchi Electric Works RL-62 laser Raman spectrometer (by the courtesy of Dr. Y. Koyama, Kwansei Gakuin University) with excitation of an argon ion laser line at 488.0 or 514.5 nm. The power of the laser beam was 100–300 mW. The slit width was 75–200 μm for simple molecules and 150–600 μm for phospholipid molecules. The temperature of a sample was controlled by a copper sample holder which was connected with a circulating water bath. The temperature was calibrated with a thermocouple. The frequency calibration has been done on the basis of the calibrated Raman bands of indene. Proton magnetic resonance spectra (100 MHz) were measured with a JEOL FX-100 FT NMR spectrometer (Institute for Protein Research, Osaka University).

Results

Raman Spectra of Choline Group. Choline iodide was investigated in detail as a simple model for molecules bearing choline groups. Selective isotope replacement is useful to see a mixing of vibrational modes which could be associated with a conformation. Raman spectra in the region from 600 to 1000 cm^{-1} of selectively deuterated choline iodide are shown in Figure 1 in both solid and aqueous dissolved states. For simplification of the discussion, the carbons of the choline group are designated as α, β, and γ [$HOC_\alpha H_2C_\beta H_2N^+(C_\gamma H_3)_3$]. Appearing in the region of interest are mainly Raman bands attributed to skeletal stretching vibrations. The strongest Raman band at 712 cm^{-1} can be assigned to the "totally" symmetric stretching vibration of the four C–N bonds (ν_1), and the medium intensity bands at 858, 942, and 953 cm^{-1} can be assigned to the symmetric (ν_2) and two asymmetric (ν_3, ν_4) stretching vibrations of the C–N bonds (Appendix). A band at 894 cm^{-1} cannot be associated with the C–N stretching vibrations, since it shifts by 14 cm^{-1} and becomes broader in the spectrum of the solution. In comparing the spectra of Figure 1, two prominent features are discerned: the isotope shift of the ν_1 band and the appearance of a weak band in solution. The isotope shifts of the ν_1 band are given in Table I. If we compare the isotope shifts found in the upper four spectra in Figure 1, where only one methyl or methylene group is selectively deuterated, the largest shift is observed with choline-α-CD_2 iodide. Since the α position is the furthest position from the C–N bonds, this large isotope shift implies a coupling between the C–N and CD_2 vibrations. Therefore this Raman band could be associated with the dihedral angle around the C_α–C_β bond. A weak Raman band appears in each spectrum of the aqueous solutions in the region from 700 to 800 cm^{-1}, where no Raman band can be found in the solid state. The relative intensity of the weak band with respect to that of the C–N "totally" symmetric stretching vibration is constant (Table I). This observation implies that the appearance of the weak band is caused by a change in the state of the sample from solid to solution.

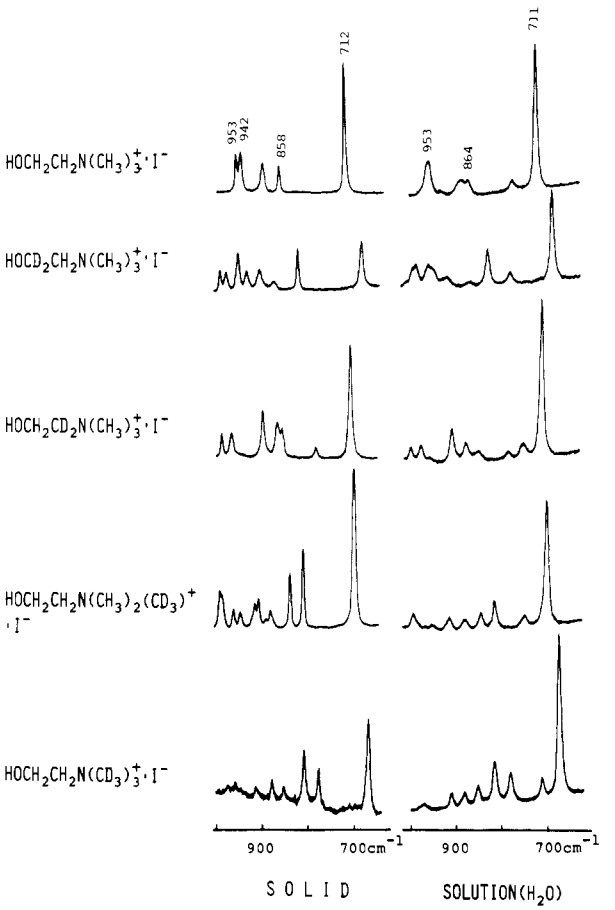

FIGURE 1: Raman spectra of nondeuterated and deuterated choline iodide in solid and aqueous solution states. The deuterated positions are shown on the left. Excitation at 488.0 nm.

Table I: Observed Frequencies of the ν_1 Band of the C–N Stretching Vibrations of Deuterated Choline Iodide and 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)[a]

	choline iodide			
deuterated position	solid (cm^{-1})	solution (at 24 °C) (cm^{-1})	rel intensity (%)	DPPC solid (cm^{-1})
nondeuterated	712	711	91	719
		765	9	
α-CD_2	676 (Δ = 36)	678 (Δ = 33)	88	682 (Δ = 37)
		771 (Δ = −6)	12	
β-CD_2	701 (Δ = 11)	704 (Δ = 7)	91	705 (Δ = 14)
		744 (Δ = 21)	9	
γ-CD_3	695 (Δ = 17)	696 (Δ = 15)	91	701 (Δ = 18)
		744 (Δ = 21)	9	
γ-$(CD_3)_3$	664 (Δ = 48)	669 (Δ = 42)	91	
		708 (Δ = 57)	9	

[a] Δ, isotope shift. The positions of α, β, and γ are shown in the text. The relative intensity is the integrated one.

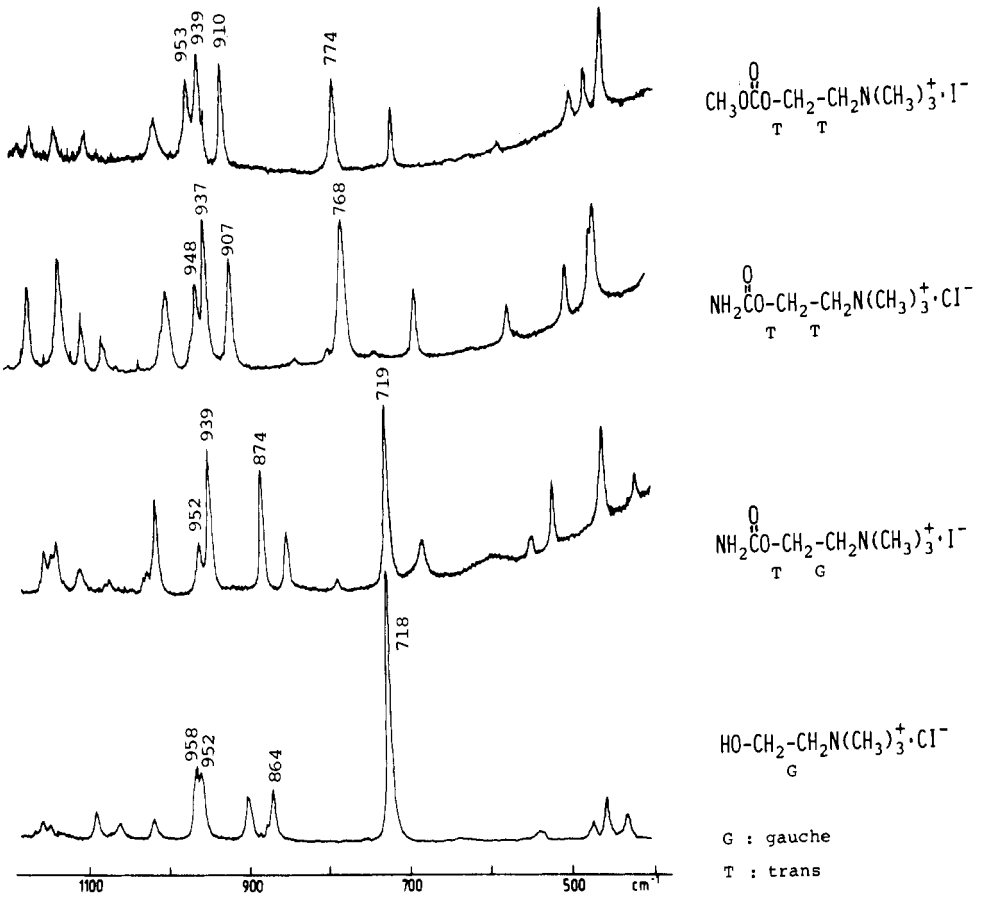

FIGURE 2: Raman spectra of choline chloride, carbamoylcholine iodide, carbamoylcholine chloride, and methoxycarbonylcholine iodide (from the bottom to the top) in the solid state. The chemical structures and conformations of the backbones as determined by X-ray analyses are shown on the right. Excitation at 488.0 nm.

So that a correlation between the Raman spectrum and a molecular structure could be established, Raman spectra of those substances whose molecular structures had been determined previously by X-ray diffraction were investigated. The spectra of choline chloride (ChCl), carbamoylcholine iodide (CarChI), carbamoylcholine chloride (CarChCl), and methoxycarbonylcholine iodide (MetChI) in crystal are shown in Figure 2 along with the chemical structures and the conformations determined by X-ray crystallography [ChCl, Senko & Templeton (1960) and Hjortås & Sørum (1971); CarChI and CarChCl, Jensen (1975); MetChI, Jensen (1979)]. As can be seen in this figure, the spectrum from 700 to 1000 cm^{-1} shows a close correlation with the conformation of the choline group. While the pattern in the region of interest is similar for the carbamoylcholine chloride and methoxycarbonylcholine iodide (for the different compounds), it is quite different for the carbamoylcholine iodide and carbamoylcholine chloride (for the same organic compounds). Specifically, Raman bands at about 720 and 870 cm^{-1} which were assigned to the C–N "totally" symmetric stretching (ν_1) and symmetric stretching (ν_2) vibrations, respectively, can be seen only for the gauche conformation of the O–C–C–N^+ backbone. In the spectra of the trans conformation, they are likely to shift to about 770 and 910 cm^{-1}, respectively. In contrast to this case, the bands assigned to the C–N asymmetric stretching vibrations (ν_3, ν_4) do not change appreciably. The same correlation was found between the solid and aqueous solution of carbamoylcholine chloride and methoxycarbonylcholine iodide. As shown in Figure 3, new Raman bands at 722 and 878 cm^{-1} (CarChCl) or 719 and 878 cm^{-1} (MetChI) appear in the spectrum of the solution in accordance with the disappearance of the bands at 768 and 907 cm^{-1} (CarChCl) or 774 and 910 cm^{-1} (MetChI). Proton magnetic resonance (^1H NMR) spectra (100 MHz) of the solutions were also examined (data are not shown). The carbamoylcholine chloride solution gives rise to essentially the same spectrum as that of the carbamoylcholine bromide solution reported earlier (Conti et al., 1971). Thus most of the O–C–C–N^+ backbones take the gauche conformation in solution as concluded for carbamoylcholine bromide solution. The ^1H NMR spectrum of the methoxycarbonylcholine iodide solution leads to the same conclusion. Again both Raman bands at about 720 and 870 cm^{-1} are associated with the gauche conformation.

For confirmation of the assignments of the Raman bands at about 770 and 910 cm^{-1} in the trans conformation, the spectra of MetChI and *N*-(deuteriomethyl)methoxycarbonylcholine iodide were compared in solid and solution (Figure 3). In the spectrum of the solid MetChI, there are only two candidates for the ν_1 mode vibration, namely, the bands at 775 and 704 cm^{-1}. The isotope shift of the band at 775 cm^{-1} is 21 cm^{-1} while that of the other one is only 4 cm^{-1}. The band at 719 cm^{-1} in solution, which was assigned to the ν_1 mode vibration, also shows a 19-cm^{-1} isotope shift. Since the isotope shift is comparable for the bands at 775 cm^{-1} in solid and at 719 cm^{-1} in solution, the band at 775 cm^{-1} can be assigned to the C–N "totally" symmetric stretching vibration (ν_1). It follows also that the band at 910 cm^{-1} should be assigned to the symmetric stretching vibration (ν_2). Unfortunately it was not possible to confirm this directly by the use of an isotope shift, since the spectrum of deuterated MetChI is very complicated in this region.

It can be concluded that the C–N stretching vibrations are not pure and their frequencies depend upon the conformation of the O–C–C–N^+ backbone. The conformation dependencies

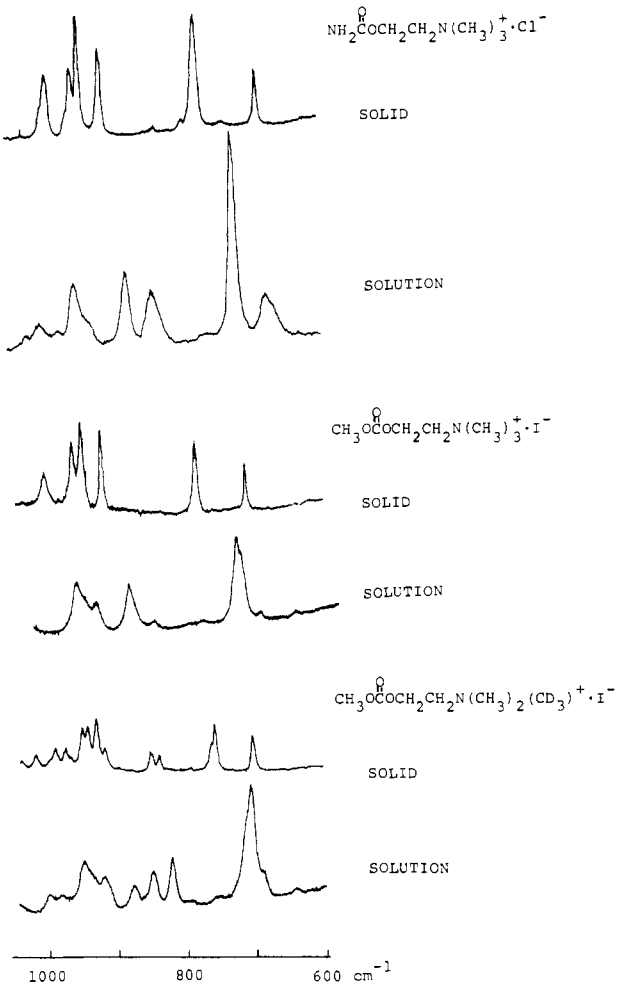

FIGURE 3: Raman spectra of carbamoylcholine chloride, methoxycarbonylcholine iodide, and methoxycarbonylcholine-*N*-CD_3 iodide (from the top to the bottom) in the solid and aqueous solution states. The chemical structures are shown on the right. Excitation at 488.0 nm.

Table II: Conformation Dependence of the Raman Bands Due to the C-N Stretching Vibrations (cm^{-1})

	conformation of O-C-C-N^+	
assignments	gauche	trans
ν_1 (sym str)[a]	~720	~770
ν_2 (sym str)[a]	~870	~910
ν_3, ν_4 (asym str)[a]	~950	~950

[a] Sym str, symmetric stretching; asym str, asymmetric stretching.

of the Raman bands are summarized in Table II. On the basis of these assignments, the small peak which appeared in the spectra of the choline iodide solution (Figure 1) can be ascribed to the ν_1 band in the trans conformation. Therefore a small population of trans conformers of the choline molecule appears in solution.

Raman Spectra of Choline Groups in Phosphatidylcholine Bilayers. To see if this simple rule can be extended from simple molecules to a more complex molecule such as phosphatidylcholine, Raman spectra of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) selectively deuterated in the choline group were investigated. Spectra from 600 to 1200 cm^{-1} are given in Figure 4 along with that of nondeuterated DPPC. A rather strong Raman band appears at 719 cm^{-1} for nondeuterated DPPC. It can be ascribed to the ν_1 band of the

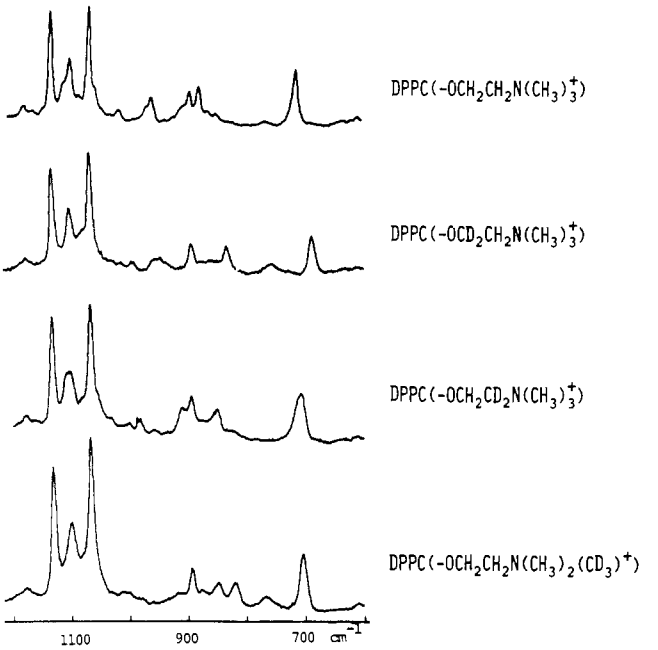

FIGURE 4: Raman spectra of nondeuterated and deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine in solid. The deuterated positions are shown on the right. Excitation at 488.0 nm.

C–N stretching vibrations. The extent of the isotope shift of the Raman band is summarized in the last column of Table I. The direction and magnitude of the isotope shift of the DPPC ν_1 band were the same as observed with choline iodide. Again the largest isotope shift was found in the case of DPPC-α-CD_2. Furthermore, the other components of the C–N stretching vibrations[2] are also found at 878 and 956 cm^{-1}. It appears, consequently, that the rule shown in Table II is valid not only for simple molecules but also for complex molecules. It follows that most of the O–C–C–N^+ backbone in the choline groups of DPPC are in the gauche conformation in the solid state. A weak band at about 770 cm^{-1} can be seen in the spectrum of nondeuterated DPPC. The Raman band comes from the stretching vibration of the esterified P–O bonds (Akutsu & Kyogoku, 1975). Because of its appearance, however, the existence of the trans conformation cannot be completely ruled out. The head-group conformation deduced from the Raman spectrum is also consistent with the conformation found in the crystal structures of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (Pearson & Pascher, 1979) and 3-lauroylpropanediol-1-phosphocholine (Hauser et al., 1980b).

The conformation of the choline group in DPPC bilayers was examined in the gel and liquid-crystalline states by using the results shown in Table II. Raman spectra of DPPC in the presence of excess water at 24 and 50 °C are shown in Figure 5. The phase change is evident from the spectral pattern from 1000 to 1200 cm^{-1}. Nevertheless, the Raman band attributed to the ν_1 mode vibration stays at 717 cm^{-1} irrespective of the phase change. This is also the case for the 875-cm^{-1} band. This observation indicates that most of the choline groups assume the gauche conformation at the O–C–C–N^+ bond not only in the gel state but also in the liquid-crystalline state.

Raman Spectra of Sphingomyelin Bilayers. Bovine brain sphingomyelin is known to form a bilayer structure and to show

[2] The band at 878 cm^{-1} could overlap a band contributed by the diacylglycerol modes, since solid dipalmitin gives two Raman bands at 892 and 881 cm^{-1}. However, the band of interest shifts in the case of DPPC-α-CD_2, and no sharp band remains at the original position (Figure 4). Accordingly, the assignment is justified.

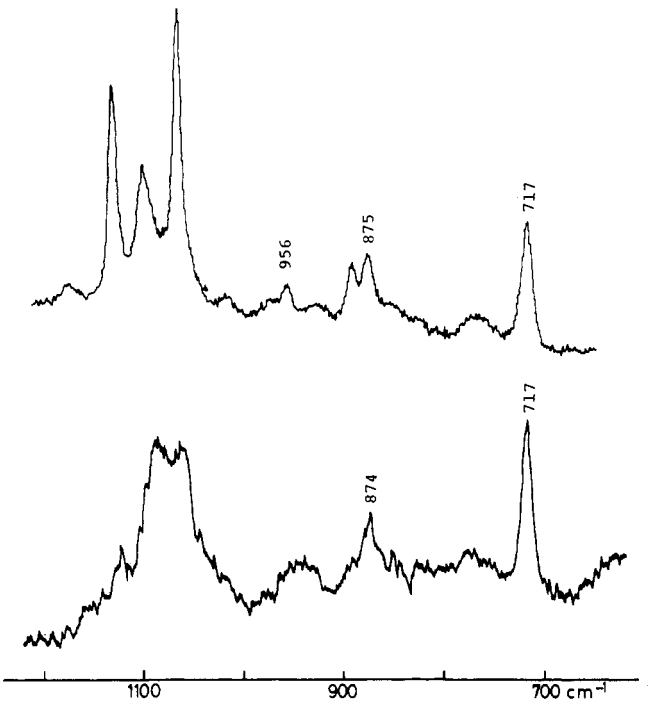

FIGURE 5: Raman spectra of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine in the presence of excess water at 24 (upper) and 50 °C (lower). Excitation at 488.0 nm.

a broad phase transition at 20–45 °C (Barenholz et al., 1976; Hui et al., 1980). Raman spectra of the sphingomyelin in the solid, the gel (at 20 °C), and the liquid-crystalline (at 54 °C) states are shown in Figure 6. In all spectra, the Raman bands at about 720, 875, and 960 cm^{-1} can be found. Especially the band at 720 cm^{-1} is quite strong. It can be concluded that most of the choline groups of sphingomyelin assume the gauche conformation in the bilayer structure above as well as below the phase transition.

Discussion

The conformational assignments of the Raman bands of the C–N stretching vibrations have been established in this work. This conformational dependence of the frequency is in good agreement with the known rules for *n*-propane derivatives. The frequency of the C–X stretching vibration (X = Cl, S) of *n*-propane derivatives remarkably depends upon the conformation (Mizushima et al., 1957; Shipman et al., 1962; Sugeta et al., 1972). Normal coordinate analyses suggest that the C–C–C bending vibration couples with the C–X stretching in the trans conformation but not in the gauche conformation (Snyder & Schachtschneider, 1969; Sugeta, 1975). The coupling gives rise to the high frequency shift of the C–X stretching vibration by about 70 cm^{-1}. Therefore, the higher frequencies of the ν_1 and ν_2 bands in the trans rather than in the gauche conformation can be ascribed to vibrational mixing with the O–C–C bending vibration. The largest isotope shift was observed for the choline-α-CD_2 group in the gauche conformation, suggesting that the C–N stretching vibrations of the gauche conformation couple with the α-CH_2 rocking vibration. A correlation between the C–N stretching vibrations and the conformation of the O–C–C–N^+ backbone was suggested on the basis of a normal coordinate analysis (Fringeli, 1981). Qualitatively, our results are in accordance with the suggestion.

The gauche conformation appears to be more stable in the O–C–C–N system in general. The trans conformation becomes stable only in some crystals, which must be attributed

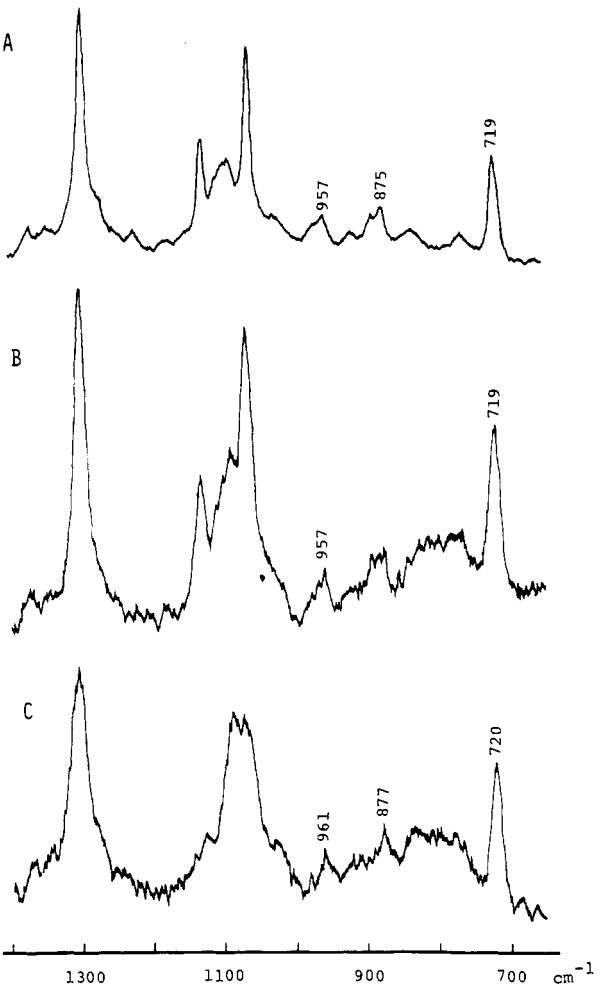

FIGURE 6: Raman spectra of bovine brain sphingomyelin in solid (A) and in the presence of excess water (B, C; B, at 20 °C; C, at 54 °C). Excitation at 514.5 nm.

to the intermolecular interactions. The energy difference between the gauche and trans conformations of choline iodide could not be estimated from the temperature-change experiments because the change of the intensity of each Raman band was too small. If we assume the intrinsic Raman intensity of the ν_1 band is the same for the gauche and trans conformations and the fraction of the trans is 10% ± 2% at 24 °C (Table I), the energy difference (ΔE) between the trans and gauche conformations can be calculated to be 0.9 ± 0.1 kcal/mol. Actually the intrinsic intensity of the Raman band at about 770 cm^{-1} is obviously weaker than that at about 720 cm^{-1} (Figure 2). Therefore the real ΔE should be smaller than 0.9 kcal/mol. ΔE estimated for phosphoethanolamine by ^1H NMR was 1.3 ± 0.3 kcal/mol (Akutsu & Kyogoku, 1977). The gauche conformation of phosphocholine was pointed out to be more stable than that of phosphoethanolamine on the basis of spin analysis. Accordingly it can be concluded that the introduction of the phosphate group leads to the stabilization of the gauche conformation of the choline group.

Reasons why the gauche conformation is more stable may be found in the electrostatic interaction between the oxygen atom and a positive charge at the nitrogen atom (Sundaralingam, 1968; Hauser et al., 1980a). However, this interaction cannot be the major determinant since the gauche conformation is still stable in phosphoethanolamine at pH 9.95 (Akutsu & Kyogoku, 1977). It should be explained by a more general mechanism which causes so-called gauche effect in many structurally similar molecules, both organic and inorganic, containing pairs of electronegative atoms or highly polar

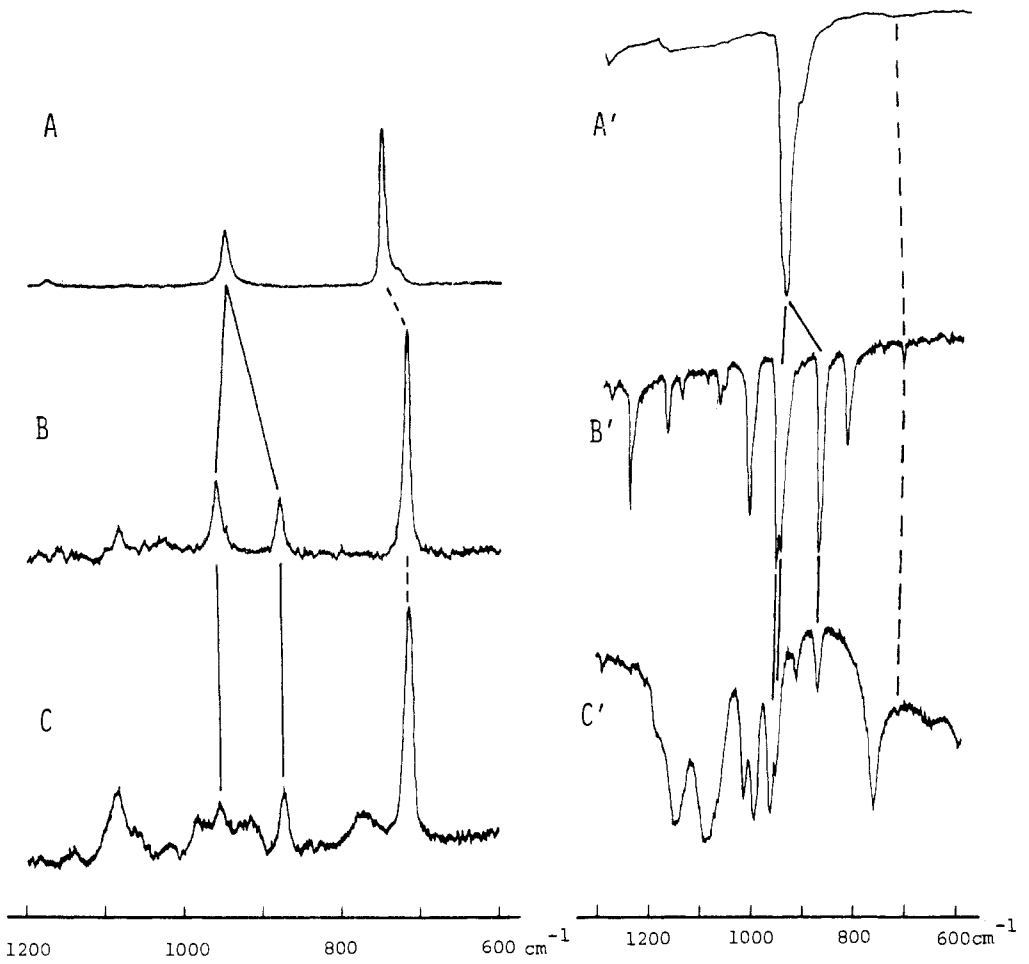

FIGURE A1: Raman (A, B, C) and infrared (A′, B′, C′) spectra of tetramethylammonium bromide (A, A′), ethyltrimethylammonium iodide (B, B′), and phosphocholine–calcium chloride (C, C′). (A, B, C) Aqueous solution; (A′, B′, C′) solid. Excitation of Raman scattering was at 488.0 nm for (A) and 514.5 nm for (B) and (C). (Broken line) Originated from A_1 species of T_d symmetry; (solid line) originated from F_2 species of T_d symmetry.

bonds (Wolfe, 1972). A recent INDO calculation suggested that the primary contribution to the stabilization of the gauche conformation comes from vicinal interactions between orbitals of bond and antibond types (Brunck & Weinhold, 1979). Electrostatic interactions and steric effect would be of second importance.

The comparison of the Raman spectra of the model compounds with those of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and sphingomyelin from bovine brain showed that the gauche conformation is dominant in the choline group of DPPC and sphingomyelin bilayers in the liquid-crystalline state as well as in the gel state. This is the first direct evidence for the gauche conformation of the choline group in the gel and in the liquid-crystalline states. In phosphatidylcholine bilayers, a gauche conformation has been inferred on the basis of deuterium and phosphorus NMR (Seelig et al., 1977) or ^{1}H NMR (Akutsu & Kyogoku, 1977; Hauser et al., 1980a) studies. From the NOE experiments of phosphorus NMR, the orientation of the phosphocholine groups in the sphingomyelin bilayers was suggested to be similar to those in the phosphatidylcholine bilayers (Yeagle et al., 1977). Therefore, it can be also said that both conformation and orientation of the phosphocholine in the bilayer are quite similar for phosphatidylcholine and sphingomyelin. The phase transition temperatures of *N*-palmitoylsphingomyelin and *N*-stearoylsphingomyelin are also known to be similar to those of dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine, respectively (Barenholz et al., 1976; Estep et al., 1980). It implies that the structural difference in the backbone groups (sphingosine and glycerol) does not affect much the intermolecular interaction energy as well as the structure and orientation of the polar head groups.

Table AI: Frequencies of the C–N Stretching Vibrations of Phosphocholine (cm^{-1})[a]

assignments		Raman		infrared
		solution	solid	solid
sym str (A_1 of local C_{3v})	ν_1	716 ($\rho < 0.01$)	720 (s)	716 (vw)
sym str (A_1 of local C_{3v})	ν_2	874 ($\rho = 0.52$)	876 (m)	873 (w)
asym str (E of local C_{3v})	ν_3	956	956 (m)	954 (m)
	ν_4		969 (w)	965 (s)

[a] ρ, depolarization ratio; sym str, symmetric stretching; asym str, asymmetric stretching; s, strong intensity; m, medium intensity; w, weak intensity; vw, very weak intensity.

Since the Raman band at about 720 cm^{-1} is relatively strong, this could be useful in the study of the structure of phosphatidylcholine and sphingomyelin in biomembranes. This Raman band has been observed previously in erythrocyte ghosts (Wallach & Verma, 1975) and sarcoplasmic reticulum membranes (Milanovich et al., 1976), suggesting that the gauche conformation is also predominant in biomembranes. The general significance of these conformation-sensitive Raman bands is not restricted to the membrane field. It must be a powerful tool to investigate the structure of acetylcholine and its interaction with acetylcholine receptors in situ. For a quantitative work, the use of choline-γ-$(CD_3)_3$ group is recommended because the band due to the C–N "totally" sym-

metric stretching vibration (ν_1) does not overlap other bands in both trans and gauche conformations.

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Appendix

Assignments of the Stretching Vibrations of the C–N Bonds of the Choline Group. The assignments of the Raman bands attributed to the C–N stretching vibrations of the choline group are not yet well established. For example, although the Raman band of phosphatidylcholine at about 720 cm^{-1} is usually assigned to the symmetric stretching vibration of the C–N bonds (Brown et al., 1973), it is assigned to the CH_2 rocking vibration in connection with acetylcholine and its analogues (Aslanian et al., 1974, 1977). Since the frequencies of the C–N stretching vibrations of the choline group turned out to be conformation sensitive in this work, the assignments have been established by comparison with model molecules.

Raman (for aqueous solution) and infrared (for solid) spectra of tetramethylammonium bromide, ethyltrimethylammonium iodide, and phosphocholine–calcium chloride (pH 4.0, adjusted by HCl) are shown in Figure A1. The Raman spectrum of tetramethylammonium ion and its assignments were reported by Edsall (1937). This molecule has T_d symmetry. The band at 753 cm^{-1} is strongly polarized and was assigned to the totally symmetric stretching vibration of the C–N bonds (A_1 species). It must be infrared inactive. The band at 952 cm^{-1} was assigned to the triply degenerate C–N stretching vibration (F_2 species). The infrared spectrum also supports the assignments. In the case of ethyltrimethylammonium ion, the symmetry decreases to C_s. Since the quaternary ammonium group has local C_{3v} symmetry, the C–N stretching vibration should reveal four Raman bands, namely, two symmetric (A_1 species of C_{3v} symmetry) and a couple of degenerate-like (E species of C_{3v} symmetry) stretching vibrations. We denote the former vibrations as ν_1, ν_2 modes and the latter vibrations as ν_3, ν_4 modes. In the case of the ν_1 mode, all of the four C–N bonds vibrate in phase. The vibrational modes of ν_1 and ν_2 are different only in the phase of the stretching vibration of the C–N bond of the *N*-ethyl group. ν_1 originates from A_1 species of T_d symmetry, and ν_2, ν_3, and ν_4 originate from F_2 species of T_d symmetry. For clarification of the nature of the vibrational mode, ν_1 is referred to as the "totally" symmetric stretching vibration in the text. In the Raman spectrum of ethyltrimethylammonium aqueous solution, three relatively strong Raman bands are observed in the region of interest. The strongest one at 719 cm^{-1} can be easily assigned to the ν_1 mode vibration. Actually it is strongly polarized ($\rho < 0.01$; ρ = depolarization ratio). A very weak infrared band at 714 cm^{-1} also supports this assignment. The band at 959 cm^{-1} is depolarized ($\rho = 0.73$) and splits to doublet in solid (at 953 and 961 cm^{-1}; the spectrum is not shown). That is also the case in the infrared spectrum as can be seen in Figure A1, part B′). Therefore the band at 959 cm^{-1} can be assigned to the degenerate-like (asymmetric) stretching vibration, namely, ν_3 and ν_4 modes. Since the band at 878 cm^{-1} is polarized ($\rho = 0.58$), it can be assigned to the ν_2 mode of the symmetric stretching vibration. These three bands (four in solid) appear at similar positions in the Raman and infrared spectra of phosphocholine (parts C and C′ of Figure A1). Depolarization ratios of these bands are also similar to those of ethyltrimethylammonium solution except for the band at 956 cm^{-1}. Its depolarization ratio could not be determined because of a strongly polarized band in the vicinity. Frequencies and assignments for phosphocholine are given in Table AI along with the depolarization ratios. The assignments hold true for other choline groups as can be seen in the text. The fact that the frequencies of the C–N stretching vibrations of ethyltrimethylammonium ion are similar to those of the choline group in the gauche conformation can be accounted for as follows. Because of vibrational mixing, the frequencies of the C–N vibrations depend upon the atom species located at the trans position with respect to nitrogen (N–C–C–X) (Mizushima et al., 1957; Shipman et al., 1962; Sugeta et al., 1972). In the case of the gauche conformation of the O–C–C–N^+ system, the position is occupied by a proton. Since the atom at that position of ethyltrimethylammonium ion is always a proton, the frequencies should be similar to those of the O–C–C–N^+ system in the gauche conformation.

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Interaction of Metal Ions with Phosphatidylcholine Bilayer Membranes†

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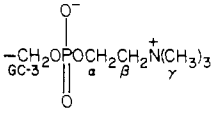
ABSTRACT: The interaction of mono-, di-, and trivalent metal ions with bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was investigated with deuterium and phosphorus magnetic resonance. With selectively deuterated lipids the measurements of the residual deuterium quadrupole splitting provided a sensitive handle to monitor directly the binding of ions, including the weak binding of Na^+ or $(CH_3)_4N^+$. For the α segment of the choline group ($-NCH_2CD_2O-$) changes in the quadrupole splitting of up to 9 kHz were observed. All measurements were made with nonsonicated DPPC dispersions. The ion concentrations were varied between 5 mM and 2 M, an almost 50-fold larger concentration range than accessible with nuclear magnetic resonance shift reagents. From a systematic comparison of various ions the following conclusions could be derived. (1) Addition of metal ions led to a structural change at the level of the polar groups. The glycerol backbone or the beginning of the fatty acyl chains was not affected. (2) The strength of interaction increased with the charge of the metal ion in the order $Na^+ < Ca^{2+} < La^{3+}$. However, distinct differences were also noted between ions of the same charge. Furthermore, the strongly hydrophobic tetraphenylammonium ion induced almost the same change as La^{3+}. (3) The variation of the quadrupole splittings with ion concentration exhibited a plateau value at high concentrations of La^{3+}. The titration curves of DPPC with Ca^{2+} and La^{3+} could be described in terms of a Langmuir adsorption isotherm with an interaction potential. Apparent binding constants of $K_{LaCl_3} \simeq 120\ M^{-1}$ and $K_{CaCl_2} \simeq 19\ M^{-1}$ were derived. (4) The addition of NaCl considerably enhanced the binding of Ca^{2+} and La^{3+}, apparently without affecting the plateau value of the quadrupole splitting. (5) The ion-induced conformational changes were qualitatively similar for all ions investigated. The various binding data could be summarized by plotting the quadrupole splittings of the α segment ($-OCD_2CH_2N-$) vs. those of the β position ($-OCH_2CD_2N-$). This plot yielded a straight line comprising all ions and concentrations investigated except Eu^{3+}. The quadrupole splittings of DPPC observed in the presence of chloroform or cholesterol and the variation of the quadrupole splittings with temperature could also be summarized in a linear plot that was different from that obtained for metal ion binding. This suggests the existence of at least two kinds of structural responses of the polar head groups to external perturbations.

Phosphatidylcholine is one of the predominant phospholipids in membranes, and a large fraction of most membrane surfaces is occupied by phosphocholine groups. The interactions of metal ions with the uncharged phosphatidylcholine bilayer can be expected to be relatively weak compared to those with negatively charged lipids such as phosphatidylglycerol or phosphatidylserine. Nevertheless, even small changes in the head-group orientation and flexibility could significantly alter the electrical properties of the membrane surface, producing, in turn, changes in the physiological or biochemical characteristics of the membrane. Thus, the problem of metal ion binding to phosphatidylcholine bilayers has attracted much attention, and a variety of methods have been employed [for a review, see Hauser & Phillips (1979)]. Deuterium magnetic resonance is a particularly promising method in this respect since quite large changes in the residual deuterium quadrupole splittings can be induced by the interaction with ions (Brown & Seelig, 1977). In the present study this effect has been exploited more systematically, and results on the concentration, temperature, and ion dependence are provided. A set of phosphatidylcholine molecules selectively deuterated in the choline moiety was employed in order to analyze separately the changes of the various head-group segments, including the glycerol backbone. The movement of the phosphate group was followed by phosphorus magnetic resonance.

Materials and Methods

For simplification of discussion the following nomenclature is employed for the glycerol backbone and the phosphocholine head-group segments:



1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)[1] was

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